4) preparing extraction buffer without salt identified as buffer B,

5) preparing extraction buffer with salt identified as buffer C,

6) preparing cytoplasmic extraction clarification buffer identified as buffer D,

7) suspending and maintaining the cells in buffer A for approximately 15 min,

8) centrifuging at approximately 2,000 rpm for approximately 5 min at approximately 4 °C,

9) removing the upper cytoplasmic supernatant fraction and clarifying this fraction by adding buffer D at approximately 4 °C followed by centrifuging at approximately 13,000 rpm for approximately 15 min,

10) removing the top clear supernatant, quick freezing on dry ice and storing at approximately -86 °C until assayed,

11) washing the bottom nuclear fraction from step 8 with buffer B and centrifuging at approximately 2,000 rpm for approximately 5 min at approximately 4 °C,

12) suspending the pelleted nuclei in buffer C on ice,

13) tapping the mixture of step 12 for approximately 45 min to extract the nuclear proteins,

14) centrifuging the mixture of step 13 at approximately 13,000 rpm for approximately 15 min at approximately 4 °C,

15) removing the top clear supernatant, quick freezing on dry ice in aliquotes and storing at approximately -86°C,

16) storing the remaining bottom nuclear fraction containing nucleic acids from step 14 at approximately -86 °C,

17) performing DNA extraction,

3. (Newly submitted) The method of claim 2 wherein the step of performing DNA extraction comprises the steps of:

1) adding buffer containing Tris-HCl, EDTA, NaCl and SDS to the nuclear fraction of step 16,
2) adding RNAase A followed by proteinase K for approximately 2 hours at approximately 37 °C with gentle tapping,
3) mixing with an equal volume of Tris-HCl and EDTA buffer saturated phenol,
4) centrifuging at approximately 3,000 rpm for approximately 10 min, and collecting the upper aquous phase containing DNA,
5) mixing with equal volume of phenol and chloroform,
6) centrifuging at approximately 3,000 rpm for approximately 10 min, and collecting the upper aquous phase containing DNA,
7) mixing with an equal volume of chloroform/isoamyl alcohol prepared at a ratio of 96:4,
8) centrifuging at approximately 3,000 rpm for approximately 10 min,
9) collecting the upper aquous phase containing DNA and precipitating using a salt and ethanol,
10) cenrifuging at approximately 13,000 rpm for approximately 30 min,
11) removing the upper liquid phase and air drying the bottom DNA pellet,
12) dissolving the air dried DNA pellets in Tris-HCl and EDTA buffer.

4. (Newly submitted) The method of claim 2 in which after step 15 further includes the step of analyses of the biologically active transcription factors comprising the steps of:

1) synthesizing single stranded oligonucleotides and annealing with the complimentary strand,
2) preparing probes by radiolabeling annealed oligonucleotide with γ-^{32}P-ATP, T4 polynucleotide kinase, and kinase buffer,

3) filling in the 5' over-hang ends with Klenow, Klenow buffer and 0.15 mM each of dATP, dCTP, dGTP, and dTTP,

4) purifying the labeled oligonucleotides from the unincorporated nucleotides,

5) precipating the labeled, purified oligonucleotides with ethanol,

6) resuspending the labeled oligonucleotide probes in sterile Tris-HCl and EDTA buffer,

7) performing sequence specific DNA-protein binding analysis by gel electrophoretic mobility shift assay,

8) treating the protein extract with specific antibodies prior to the addition of γ-^{32}P-ATP labeled oligonucleotide probe for supershift assays,

9) separating the DNA-protein bound complexes on a acrylamide with bis gel,

10) vacuum drying the gel with heat and autoradiography.

5. (Newly submitted) The method of claim 2 wherein the step of preparing cell lysis buffer identified as buffer A comprises:

preparing 20 mM Hepes, pH 7.9, 10 mM NaCl, 3 mM $MgCl_2$, 0.1% NP-40, 10% glycerol, 0.2 mM EDTA, 1 mM dithiothreitol, 0.4 mM phenylmethylsulfonyl fluoride, 1 μg/ml antipain, 1 μg/ml leupeptin.

6. (Newly submitted) The method of claim 2 wherein the step of preparing extraction buffer without salt identified as buffer B comprises:

preparing 20 mM Hepes, pH 7.9, 0.2 mM EDTA, 20% glycerol, 1 mM dithiothreitol, 0.4 mM phenylmethylsulfonyl fluoride, 1 μg/ml antipain , 1 μg/ml leupeptin.

7. (Newly submitted) The method of claim 2 wherein the step of preparing extraction buffer with salt identified as buffer C comprises: